Claims

What is claimed is:

1. A guide oligonucleotide comprising single-stranded or partially double-stranded nucleic acid, which comprises: target complementary region, constant region, identifier sequence, at least one restriction site.

2. The guide oligonucleotide of claim 1, wherein said at least one restriction site comprises first and second restriction sites which are different, wherein said second restriction site is adjacent to said constant region.

3. The guide oligonucleotide of claim 1, wherein said identifier sequence is specific for each said guide oligonucleotide and is located between the first and second restriction sites.

4. The guide oligonucleotide of claim 1, wherein said constant region is located at the most 3' or 5' end of said guide oligonucleotide, wherein said constant region comprises sequence complementary or identical to an amplification primer sequence.

5. The guide oligonucleotide of claim 1 further comprising 5' or 3' end label.

6. The guide oligonucleotide of claim 5, wherein said end label comprises biotin.

7. The guide oligonucleotide of claim 1, wherein said identifier sequence and first restriction site are part of target complementary region.

8. The guide oligonucleotide of claim 1, wherein said identifier sequence and first restriction site are not part of target complementary region.

9. The guide oligonucleotide of claim 1 further comprising additional enzyme acting sequence which supports digestion of target sequence strand hybridized to said target complementary region of said guide oligonucleotide.

10. The guide oligonucleotide of claim 9, wherein said additional enzyme acting sequence comprises restriction site.

11. The guide oligonucleotide of claim 10, wherein said restriction site comprises type IIS restriction site or nicking restriction site.

12. The guide oligonucleotide of claim 11, wherein said type IIS restriction site or nicking restriction site comprise double-stranded restriction enzyme recognition sequence.

13. The guide oligonucleotide of claim 10, wherein nucleotides of the cleavage site of said restriction site on the target complementary region are modified, whereby the modified nucleotides are resistant to cleavage.

14. The guide oligonucleotide of claim 13, wherein said modified nucleotides comprise phosphorothioate linkages.

15. The guide oligonucleotide of claim 9, wherein said additional enzyme acting sequence comprises RNase H digestion sites when the target is RNA.

16. The guide oligonucleotide of claim 15, wherein the target complementary region of said guide oligonucleotide comprises chimeric RNA and DNA.

17. A set of guide oligonucleotides comprising multiple guide oligonucleotides each having a target specific target complementary region, a guide oligonucleotides specific identifier sequence, the same first restriction site, the same second restriction site, and the same constant region sequence.

18. A method of analyzing polynucleotides in a sample, said method comprising steps of:
(a) hybridizing guide oligonucleotides or a set of guide oligonucleotides or more than one set of guide oligonucleotides in accordance with any one of the preceding claims to target polynucleotides, whereby target complementary regions of said guide oligonucleotides become double-stranded if the target sequences are present in the sample;
(b) forming double-stranded or partially double-stranded guide oligonucleotide intermediates including double-stranded first restriction sites;
(c) digesting said double-stranded or partially double-stranded guide oligonucleotides intermediates with first restriction enzyme at the first restriction site; and
(d) analyzing the digested parts containing identifier sequences.

19. The method of claim 18, wherein the first restriction site and identifier sequence are part of the target complementary region of the guide oligonucleotide, and said step (b) is completed after said step (a).

20. The method of claim 18, wherein the target polynucleotides are RNA, and said step (b) of forming double-stranded or partially double-stranded guide oligonucleotide intermediates comprises: digesting the target RNA strand of RNA/DNA hybrid by a nuclease, extending the 3' end of the digested strand on guide oligonucleotide templates by a nucleic acid polymerase, whereby the downstream sequences 5' to the target complementary region of the guide oligonucleotide including the first restriction site become double-stranded.

21. The method of claim 20, wherein said nuclease is RNase H.

22. The method of claim 18, wherein the guide oligonucleotide comprises additional restriction site, and said step (b) of forming double-stranded or partially double-stranded guide oligonucleotide intermediates comprises: digesting target sequence strand at the restriction digestion site of said additional restriction site by a restriction enzyme, extending the 3' end of the digested strand on guide oligonucleotide templates by a

nucleic acid polymerase, whereby the downstream sequences 5' to the target complementary region of the guide oligonucleotide including the first restriction site become double-stranded.

23. The method of claim 18, wherein the target complementary regions of said guide oligonucleotides hybridize to free 3' ends of the target sequences, and said step (b) of forming double-stranded or partially double-stranded guide oligonucleotide intermediates comprises: extending said free 3' ends of the target sequences by a nucleic acid polymerase using said guide oligonucleotides as templates, whereby the downstream sequences 5' to the target complementary region of the guide oligonucleotide including the first restriction site become double-stranded.

24. The method of claim 18, wherein said step (b) of forming double-stranded or partially double-stranded guide oligonucleotide intermediates comprises: trimming single-stranded target sequence 3' to the target region hybridized to the guide oligonucleotide with an exonuclease activity, extending 3' ends of the trimmed target sequences by a nucleic acid polymerase using said guide oligonucleotides as templates, whereby the downstream sequences 5' to the target complementary region of the guide oligonucleotide including the first restriction site become double-stranded.

25. The method of claim 24, wherein said guide oligonucleotide comprises at least one modified nucleotide or modified phosphodiester linkage in at least an ultimate 3' end position to resist exonuclease activity.

26. The method of claim 18 further comprising: after said step (a) or after step (b) capturing said polynucleotides or said oligonucleotide on a solid support through the end labels, and stringency washing.

27. The method of claim 18 further comprising: after said step (c) isolating the digested parts containing identifier sequences and constant regions, wherein said digested parts are attached on solid support or in supernatant.

28. The method of claim 18, wherein said step (d) of analyzing the digested parts containing identifier sequences comprises: detecting said digested parts by mass spectrometry, electrophoresis or microarray.

29. The method of claim 18, wherein said step (d) of analyzing the digested parts containing identifier sequences comprises: ligating said digested parts to each other by a nucleic acid ligase to produce at lease one joined identifier fragment, amplifying joined identifier fragments using primers that are complementary or identical to constant regions of the guide oligonucleotides, analyzing the amplified products.

30. The method of claim 29, wherein said analyzing the amplified products comprises determining the nucleotide sequences of said amplified products.

31. The method of claim 29, wherein said analyzing the amplified products comprises: digesting said amplified products with first and second restriction enzymes to release individual identifier sequences, detecting and quantifying said identifier sequences by a detection method.

32. The method of claim 31, wherein said detection method comprises mass spectrometry, electrophoresis or microarray.

33. The method of claim 29, wherein said analyzing the amplified products comprises: digesting said amplified products with second restriction enzymes to release joined identifier fragments, ligating said joined identifier fragments to produce concatemers, determining the nucleotide sequence of identifier sequences in said concatemers.

34. The method of claim 33, wherein said determining the nucleotide sequence of identifier sequences in said concatemers comprises: cloning, sequencing and counting the numbers of identifier sequences.

35. The method according to claim 18 wherein said polynucleotide is RNA, cDNA or genomic DNA.